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Dosing Time-Dependency of the Arthritis-Inhibiting Effect of Tofacitinib in Mice

Shigeru Akiyama, Hideto To*

Medical Pharmaceutics, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama, Japan

***Corresponding Author:** Hideto To, Medical Pharmaceutics, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama, Japan, Tel.: +81-76-415-8811, Fax: +81-76-415-8811, E-mail: hidetoto@pha.u-toyama.ac.jp

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Abstract

Objective: Rheumatoid arthritis (RA) has a 24-hour rhythm with a characteristic symptom of morning stiffness, which causes pain in the joints from late night to early morning. We previously revealed that higher therapeutic effects were obtained in RA patients and RA animal models when the dosing time of anti-rheumatic drugs was chosen according to the 24-hour rhythms of cytokine and inflammatory reaction. In this study, we evaluated whether dosing with the Janus-associated kinases inhibitor Tofacitinib while accounting for biological rhythms results in higher therapeutic efficacy.

Method: After BALB/c mice were orally dosed with Tofacitinib at 5:00 or 17:00, the plasma concentrations were measured. Tofacitinib was administered at 5:00 and/or 17:00 every day to sensitize SKG mice, and the arthritis score was evaluated.

Results: The pharmacokinetics showed no clear differences between both groups. Using Tofacitinib once daily at 30 mg/kg/day, the arthritis scores in the 5:00-treated group were the lowest compared with those in the 17:00-treated and control groups on day 28. The arthritis-suppressing effect of Tofacitinib administered once daily at 5:00 (total dose: 15 mg/kg/day) was equivalent to that of dosing twice daily at 5:00 and 17:00 (total dose: 30 mg/kg/day), despite the two-fold difference in total daily dose.

Conclusion: Our findings suggest that using the optimal dosing time and dosing schedule improves the treatment of RA with Tofacitinib.

Keywords: Chronopharmacology; Tofacitinib; JAK Inhibitor; Rheumatoid Arthritis

Introduction

Rheumatoid arthritis (RA) has a 24-hour rhythm with a characteristic symptom of morning stiffness, which causes pain in the joints from late night to early morning. In RA patients, inflammatory cytokines are reported to have circadian rhythms, with high levels from late night to early morning and low levels during the day, in synchrony with the morning stiffness symptom [1, 2]. In a previous report, it was found that corticosteroids administered at night in RA patients can significantly relieve morning stiffness compared to that treated at morning which is currently practiced in the treatment of RA [3, 4]. Therefore, considering the characteristics of RA, chronotherapy is expected to be a beneficial treatment modality for RA treatment.

Previously, we measured inflammation levels and blood levels of inflammatory cytokines and found a diurnal rhythm with high levels in the light period and low levels in the dark period [5-8]. Considering these 24-hour rhythms, administering methotrexate (MTX) at times when inflammation levels and blood concentrations of pro-inflammatory cytokines were elevated showed higher arthritis suppression than administering MTX at times when these levels were decreased [5, 9]. Tacrolimus also showed greater arthritis suppression when administered to animal models of RA at times when inflammation levels and blood concentrations of inflammatory cytokines were elevated [6]. Based on these results, the circadian rhythm of inflammation levels and blood concentrations of pro-inflammatory cytokines should be considered to enhance the suppressive effects of RA therapeutic agents.

Janus-associated kinases (JAK) comprise four tyrosine kinases that associate with cytokine receptors and are the most upstream signaling pathways that transmit extracellular cytokine stimulation to the cell nucleus. Cytokine binding to cytokine receptors results in the binding of adenosine triphosphate (ATP) to JAKs, and thus JAKs become phosphorylated and activated. The activated JAKs phosphorylate a transcription factor known as a signal transducer and activation of transcription (STAT) [10]. Many inflammatory cytokines play an important role in the progression of RA by causing immune cell proliferation and cytokine production through this JAK/STAT pathway. JAK inhibitors are promising anti-rheumatic drugs because they can inhibit multiple inflammatory signaling pathways in a single step by blocking JAKs.

In this study, we evaluated whether dosing with the JAK inhibitor Tofacitinib while accounting for biological rhythms results in higher therapeutic efficacy.

Methods

Female SKG and BALB/c mice were purchased from CLEA Japan, Inc. and Japan SLC, Inc. The mice were kept under free eating and drinking conditions with a 12-hour light-dark cycle (light period: 7:00–19:00) and constant temperature (24 ±1°C) for at least 1 week. Animal experimental protocols were approved by the Animal Care and Use Committee at the University of Toyama (Approval number: A2021PHA-23) and conducted in accordance with their Institutional Animal Experiment Handling Rules.

BALB/c mice were orally dosed with Tofacitinib. Blood was collected at different times (0.083, 0.5, 1, 2, 4, and 8 hours), and plasma was obtained. Plasma concentrations of Tofacitinib were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Waters Corporation) [11]. The area under the curve (AUC) was calculated using the trapezoidal method.

Mannan was dissolved in PBS (+) to a concentration of 100 mg/mL. Mannan solution (0.2 mL) was intraperitoneally administered to SKG mice. Tofacitinib was suspended in 0.5% methylcellulose/0.025% Tween 20 solution at a dose volume of 0.2 mL/10 g and orally administered at 5:00 and/or 17:00 every day until day 27 from the day after Mannan sensitization (day 1). In a previously reported basic study, it was found that the circadian rhythms of inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α were highest at 9:00 and lowest at 21:00 [6]. In addition, considering these rhythms, MTX and mizoribine dosing at 5:00, when inflammatory cytokines begin to increase, were remarkably effective in experimental animals [5, 8]. On the other hands, these treated at 17:00 when inflammatory cytokines were at a low level during the day, showed almost no effect. Since Tofac-

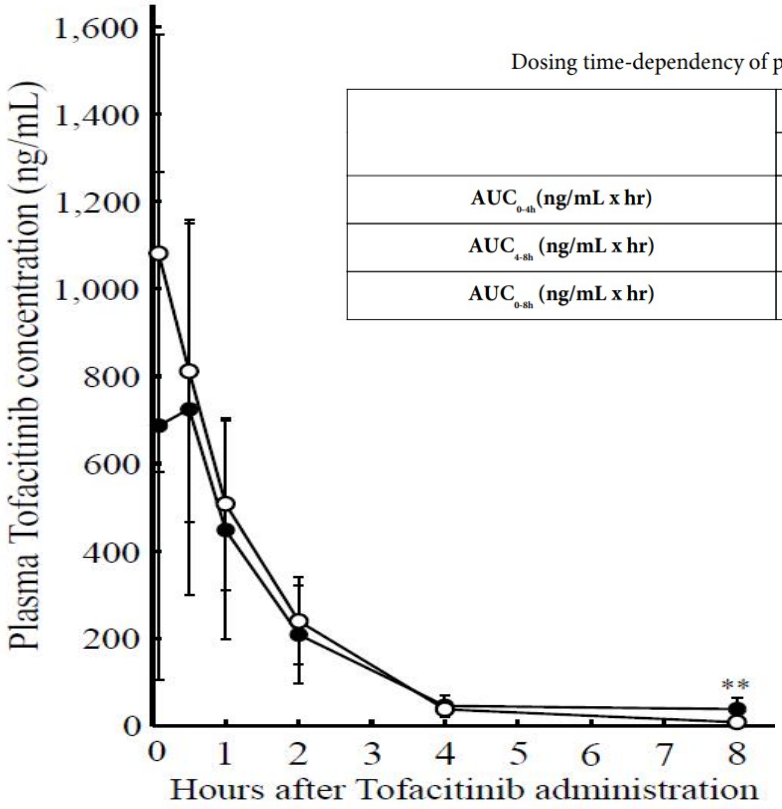
itinib has the mechanism to inhibit the production of cytokines, similar to previously reported drugs, we thought it would be beneficial to evaluate the efficacy of Tofacitinib at these dosing times and selected 5:00 and 17:00 as the dosing times.

The arthritis score was evaluated with a maximum of 5.8 points per mouse, referring to the evaluation method of Sakaguchi and coworkers [12].

All data were recorded as the mean ± standard deviation (S.D.). Differences between the two groups were analyzed using the Student's t-test. Groups were compared by one-way analysis of variance and differences between groups were determined using Scheffe's test. A probability level of less than 0.05 was considered to be significant.

Result

At 8 hours, plasma concentrations in the 5:00 dosing group were significantly higher than those in the 17:00 dosing group ($P < 0.01$; Figure 1). $AUC_{4\text{-}8h}$ was approximately twice as high in the group treated at 5:00 compared with that at 17:00 ($P < 0.01$). The $AUC_{0\text{-}8h}$ was 1,282 ± 606 ng/mL x hour in the 5:00-treated group and 1,393±439 ng/mL x hour in the 17:00-treated group, showing no significant differences between both groups. Using Tofacitinib once daily at 30 mg/kg/day, the 5:00-treated group had significantly inhibited arthritis scores compared with the control groups at all measurement points ($P < 0.05$ and $P < 0.01$; Figure 2 Left). However, the change in arthritis score of the 17:00-dosing group was lower than that of the control group, but not significantly different. On day 28, the arthritis score in the 5:00-treated group was approximately half of that observed in the 17:00-treated group.



Dosing time-dependency of pharmacokinetic parameters of Tofacitinib

	Dosing time	
	5:00	17:00
$AUC_{0\text{-}4h}$ (ng/mL x hr)	1,128 ± 576	1,322 ± 439
$AUC_{4\text{-}8h}$ (ng/mL x hr)	153 ± 56	71 ± 28**
$AUC_{0\text{-}8h}$ (ng/mL x hr)	1,282 ± 606	1,393 ± 439

**: $P < 0.01$

Figure 1: Influence of the dosing time of Tofacitinib on plasma concentrations in BALB/c mice. Tofacitinib (15 mg/kg) was orally administrated once daily at 5:00 (closed circle) or 17:00 (open circle). Each value represents the mean±S.D. (n = 9–10). **: $P < 0.01$ (Student's t-test).

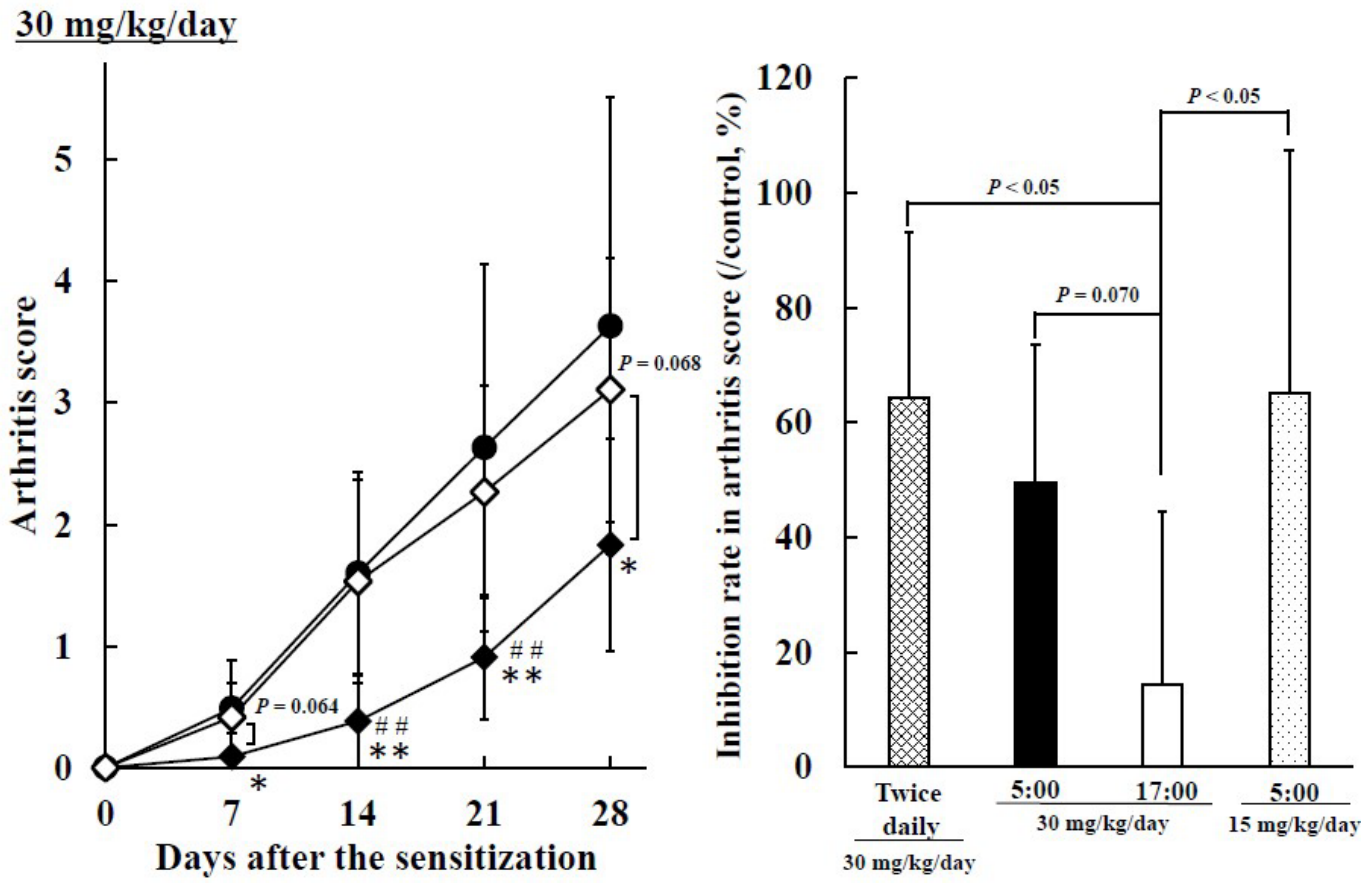


Figure 2: Influence of the dosing time of Tofacitinib on arthritis scores in SKG mice (Left). Tofacitinib (30 mg/kg) was orally administrated once daily at 5:00 (closed diamond) or 17:00 (open diamond). The 0.5% methylcellulose/0.025% Tween-20 solution was orally administrated in the control group (closed circle). Each value represents the mean±S.D. (n = 9–12). *: $P < 0.05$, **: $P < 0.01$ vs. the control group, ##: $P < 0.01$ vs. the 17:00 treated group (Scheffe's test). Influence of the dosing schedule of Tofacitinib on arthritis suppression (Right). Tofacitinib was orally administrated once (15 or 30 mg/kg/day) or twice (30 mg/kg/day) daily at 5:00 and/or 17:00. Each value represents the mean + S.D. (n = 8–12).

Furthermore, the effect of arthritis suppression differed markedly when the time of Tofacitinib dosing was considered (Figure 2 Left). Tofacitinib is orally administered twice daily in clinical practice. Therefore, we administered Tofacitinib either once or twice daily and evaluated whether the therapeutic effect differed according to the different dosing schedules.

When Tofacitinib (30 mg/kg) was administered once daily, the 17:00-dosing group exhibited significantly worse arthritis compared with the twice-daily group (total dose: 30 mg/kg/day) ($P < 0.05$; Figure 2 Right). In contrast, the 5:00-dosing group had a similar arthritis suppression rate as the twice-daily group. The arthritis scores between the 5:00 once-daily (total dose: 15 mg/kg/-day) and twice-daily (total dose: 30 mg/kg/day) groups were also similar, despite the two-fold difference in total daily dose

Discussion

Tofacitinib is mainly metabolized by hepatic metabolism, which accounts for 70% of the total metabolism and occurs through the cytochrome P450 (CYP) 3A4. In previous reports, when Tofacitinib was concomitantly administered with ketoconazole, which is a CYP3A4 inhibitor, the AUC increased approximately 2.01-fold [13]. Therefore, CYP3A4 is thought to play an important role in the metabolism of Tofacitinib. Cyp3a11 in mice functions similarly to CYP3A4 in humans. In a previous report, the protein level

of Cyp3a11 was reported to have a 24-hour rhythm with a peak from 21:00 to 1:00 and a trough at 13:00 [14]. At 8 hours after dosing, plasma Tofacitinib levels in the 5:00-dosing group were 5.15 times higher than those in the 17:00-dosing group, but when $AUC_{0\text{-}8h}$ was calculated for each dosing group, the mean values were 1,282 ng/mL x hour in the 5:00-treated group and 1,393 ng/mL x hour in the 17:00-treated group. The pharmacokinetics of the drug do not appear to be significantly different, regardless of the time of day at which the drug is administered.

We have shown in animal and clinical studies that the dosing time of anti-rheumatic drugs can impact the anti-rheumatic effect [5, 6, 9]. In MRL/lpr mice, amyloid A protein, a marker of an inflammatory response, and blood TNF-α levels displayed 24-hour rhythms with a peak at 9:00 and a trough at 5:00 after the onset of RA [7]. In the current study, SKG mice were dosed with Tofacitinib (30 mg/kg) at 5:00, when the inflammatory response and inflammatory cytokines begin to increase, and at 17:00, when they decrease. Despite the same dose, the 17:00-dosing group showed no pharmacological effect, reducing the arthritis score by an average of approximately 14.3% compared with the control group on day 28. Conversely, the 5:00-dosing group markedly suppressed arthritis exacerbations from the beginning of the treatment and reduced arthritis scores by approximately 49.6% on day 28 compared with the control group. These results demonstrated that there is an optimal dosing time associated with the 24-hour rhythm of RA symptoms that can support the effective treatment of RA.

Tofacitinib is generally taken twice a day in clinical practice. However, this study found that the 17:00-treated group showed no arthritis suppression. We evaluated the arthritis-suppressing effect of Tofacitinib using doses of 15 mg/kg once daily at 5:00 (total dose: 15 mg/kg/day), when dosing had a high efficacy, and twice daily at 5:00 and 17:00 (total dose: 30 mg/kg/day). The once-daily and twice-daily groups significantly suppressed arthritis from the early stage of medication and suppressed arthritis exacerbation by approximately 65.0% and 64.4% compared with the control group at day 28, respectively. Even though the total daily dose in the once-daily group was half of that in the twice-daily group, the arthritis suppression between the two groups was comparable. Since 17:00 is the time of day when inflammatory cytokine concentrations, such as interleukin-6, are low, Tofacitinib was not effective when administered at that time. Therefore, the once-daily and twice-daily groups showed drug effects only at 5:00, as indicated by comparable arthritis suppression. Considering the appropriate use and safety of the drugs, Tofacitinib should not be administered at dosing times when efficacy is low. Although further validation is needed, if the same arthritis suppression as twice-daily dosing can be achieved with once-daily dosing, then using the optimal dosing time may contribute not only to efficacy and safety but also to a reduction of medical costs.

Basic research has shown that small-molecule anti-RA drugs, including Tofacitinib, have high therapeutic efficacy when administered at the onset of RA exacerbations, taking into account the circadian rhythm of inflammatory cytokines and inflammatory responses unique to RA [5, 6, 8]. In RA patients, there is a clear daily rhythm in which inflammatory cytokines are elevated from late at night to early in the morning. On the other hand, there have been only a few clinical studies on chronotherapy for RA, and therefore, these studies have not yet been put into clinical practice worldwide. Since basic studies with several drugs have shown that chronopharmacology is beneficial, clinical studies with each drug should be conducted in the future to prove the usefulness of chronotherapy in RA patients.

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